

had been employed in the above experiments was left exposed in similar tubes to its influence, when a large quantity of life was rapidly developed and continued to increase. This result appears to show that the increase of life is not due to reproduction merely, but to the introduction of fresh germs; for, excepting this fresh supply, there appears to be no reason why life should increase more rapidly in the open than in the closed tubes.

In concluding this paper I have great pleasure in recognizing the able and persevering attention with which my assistant, Mr. William Thompson, has carried out these experiments.

III. "Action of Heat on Protoplasmic Life." By F. CRACE-CALVERT, F.R.S. Received May 9, 1871.

Those investigators of germ-life who favour the theory of spontaneous generation have assumed that a temperature of 212° Fahr., or the boiling-point of the fluid which they experimented upon, was sufficient to destroy all protoplasmic life, and that the life they subsequently observed in these fluids was developed from non-living matter.

I therefore made several series of experiments, in the hope that they might throw some light on the subject.

The first series was made with a sugar solution, the second with an infusion of hay, the third with solution of gelatine, and the fourth with water that had been in contact with putrid meat. The hay and putrid-meat solutions were taken because they had often been used by other investigators; sugar was employed, being a well-defined organic compound free from nitrogen, which can easily be obtained in a state of purity; and gelatine was used as a nitrogenized body which can be obtained pure and is not coagulated by heat.

To carry out the experiments I prepared a series of small tubes made of very thick and well-annealed glass, each tube about four centimetres in length, and having a bore of five millimetres. The fluid to be operated upon was introduced into them, and left exposed to the atmosphere for sufficient length of time for germ-life to be largely developed. Each tube was then hermetically sealed and wrapped in wire gauze, to prevent any accident to the operator in case of the bursting of any of the tubes. They were then placed in an oil-bath, and gradually heated to the required temperature, at which they were maintained for half an hour.

*Sugar Solution.*—A solution of sugar was prepared by dissolving 1 part of sugar in 10 parts of water. This solution was made with common water, and exposed all night to the atmosphere, so that life might impregnate it. The fluid was prepared on the 1st of November, 1870, introduced into tubes on the 2nd, and allowed to remain five days. On the 7th of November twelve tubes were kept without being heated, twelve were heated to 200° Fahr., twelve to 300°, and twelve to 400° Fahr.

The contents of the tubes were microscopically examined on the 1st of December, twenty-four days after heating.

| Sugar solution not heated.  | Heated for half an hour at 212° Fahr.  | Heated for half an hour at 300° Fahr.   | Heated for half an hour at 400° Fahr.                                    | Heated for half an hour at 500° Fahr. |
|---|--|---|--|---------------------------------------|
| There were about 30 animalcules under each field of the microscope, principally <i>small black vibrios</i> , 2 or 3 microzymes swimming slowly about, 3 or 4 <i>ordinary swimming vibrios</i> , and a few Bacteria. | A great portion of the life had disappeared, no animalcules were swimming; still this temperature had not completely destroyed life. 4 or 5 <i>small black vibrios</i> were observed moving energetically to and fro; 2 or 3 <i>ordinary vibrios</i> were also observed moving energetically in the same position of the field, that is, without swimming about. | The sugar was slightly charred, but the life was not entirely destroyed, as 1 or 2 <i>ordinary vibrios</i> and 1 or 2 <i>small black vibrios</i> were observed in motion under the field of the microscope. | The sugar was almost entirely decomposed; no trace of life was observed. | No life observed.                     |

*Remarks.*—The black vibrios here referred to are far more opaque than the other varieties of vibrios, and are the most important of all, as I have found them to resist not only very high temperatures, but all chemical solutions. I shall, in my paper on putrefaction and the action of antiseptics, describe the various vibrios and give drawings of them.

*Hay Infusion.*—An infusion of hay was made by macerating it in common water for one hour, then filtering the liquor, and leaving it exposed to the atmosphere all night, when it was sealed in the small tubes, twelve of which were used for each experiment. The infusion was made on the 4th of November, sealed in tubes on the 5th, and heated on the 7th.

The results were examined on the 1st of December, 1870, twenty-four days after being heated.

| Hay infusion not heated.  | Heated for half an hour at 212° Fahr.  | Heated for half an hour at 300° Fahr.   | Heated for half an hour at 400° Fahr.   | Heated for half an hour at 500° Fahr. |
|---|--|---|---|---------------------------------------|
| Fungus matter was observed growing on the surface of the fluids in two of the tubes. On subjecting the contents of some of the tubes to examination, from 20 to 25 animalcules were observed under each field of the microscope. This kind of life resembled small dots moving energetically to and fro; 1 or 2 ordinary vibrios were also present. | No fungus matter was noticed on the surface in any of the tubes. A few small black vibrios present in the original solution were also present in this. | No fungus matter present, but some of the small black vibrios were still present, although in less numbers. | No fungus matter observed. The fluid was filled with irregular masses of coagulated matter, and life had disappeared. | No life present.                      |

*Gelatine Solution.*—A solution of gelatine, prepared of such strength that it remained liquid on cooling, was exposed for twenty-four hours to the atmosphere. It was then introduced into the small tubes, and the tubes sealed. The solution was made on the 4th of November, the tubes sealed on the 5th, and subjected to the different temperatures on the 7th.

The fluids were examined on the 1st of December, 1870, twenty-four days after being heated.

| Gelatine solution not heated.   | Gelatine solution heated for half an hour at 100° Fahr.   | Heated for half an hour at 212° Fahr.                                     | Heated for half an hour at 300° Fahr. | Heated for half an hour at 400° Fahr. |
|---|---|---|---------------------------------------|---------------------------------------|
| There were 7 or 8 animalcules under each field, 5 or 6 of which were quite different to any thing observed in the other fluids. They had long thin bodies, swimming with a peristaltic motion. 1 or 2 ordinary swimming vibrios were also present; but the small black vibrios were absent. | Life seemed to have only slightly decreased, and none of the animalcules were swimming. The peculiar animalcule mentioned in the first column appeared to retain still its peristaltic motion, but not sufficient power to move across the field, a few ordinary vibrios being also observed moving to and fro. | A very decided diminution in the quantity of life present was noticeable. | No life present.                      | No life present.                      |

*Putrid-Meat Fluid.*—Water was placed in an open vessel, and a piece of meat suspended in it until it became putrid and contaminated with myriads of animalcules. This fluid was placed in the usual tubes, which were sealed on the 7th of November, and heated on the same day.

The contents of the tubes were subjected to examination on the 1st of December, or twenty-four days after having been heated.

| Not heated.   | Heated for half an hour at 100° F.  | Heated for half an hour at 212° F.   | Heated for half an hour at 300° F.  | Heated for half an hour at 400° F. | Heated for half an hour at 500° F. |
|---|---|--|---|------------------------------------|------------------------------------|
| A large quantity of life was present, namely, microzyma and several distinct species of vibrios, among which were a number of the small blackones frequently mentioned. | This temperature had but slightly affected the life present, the animalcules being as numerous as in the liquid not heated, and moving as usual. However, one species of very long vibrios appeared to be considerably affected, as they were much more languid in their movements. | This liquor differed from all the others in being turbid and coagulated. Life was still present; and although heat had deprived the animalcules of the power of locomotion, still they retained a sufficient amount of vital force to place it beyond a doubt that life was not destroyed. | The liquid was quite clear, the albumen (which is coagulated at 200°) appearing to be redissolved. A large quantity of the life in the fluid was destroyed, but some vibrios still remained, the small blackones being the most numerous. | All life had disappeared.          | All life had disappeared.          |

The results recorded in the above Tables show that protoplasmic life is but slightly affected by a temperature of 212° F., and that, even at a temperature of 300° F., it is not entirely destroyed, excepting in the case of gelatine. In all the other fluids a temperature of 400° F. is necessary to completely destroy the life. These experiments, therefore, clearly show that the life found by previous experimenters in fluids which have been submitted to heat was not due to heterogenesis, but to life which had remained in the fluids, as I have seen no experiment reported where the temperature to which the fluids were exposed exceeded 300° F.\*

I am the more justified in making this statement, as I have repeatedly examined the contents of tubes which had been submitted to a temperature of 400° F., both immediately after cooling and at all periods up to thirty days, and was unable in any instance to detect the slightest trace of life.

This important result corroborates those recorded in my previous paper, and proves that the spontaneous-generation theory is not yet by any means established.

\* It is with pleasure that I find these experiments to confirm the suggestion of Dr. Beale, in his work entitled "Disease-Germs, their supposed Origin," page 50 (which I read a few weeks ago), that "living forms might live though exposed, under certain conditions, to a temperature of 350° F."

It occurred to me that it might be interesting to examine the influence on pure albumen of the putrid-meat fluids that had been heated, and note whether they still possessed the property of propagating life. A solution was prepared by mixing the albumen of a new-laid egg with pure distilled water free from life (prepared as described in my previous paper). Equal volumes of this solution were placed in six small test-tubes, which had been cleansed with hot vitriol and well washed with pure water. To one tube two drops were added of the putrid-meat solution that had been heated to 100° F., to a second two drops of that heated to 212° F., to a third two drops of that heated to 300° F., to a fourth an equal bulk of fluid heated to 400° F., and to a fifth the same quantity heated to 500° F. In the sixth the albuminous solution, without any thing added, was kept for comparison.

The tubes were sealed, and kept from the 1st of February to the 9th.

#### RESULTS OF EXAMINATION.

| Albumen solution.   | Albumen solution, with putrid-meat liquor, heated to 100° F. | Albumen solution, with putrid-meat liquor, heated to 212° F. | Albumen solution, with putrid-meat liquor, heated to 300° F. | Albumen solution, with putrid-meat liquor, heated to 400° F. | Albumen solution, with putrid-meat liquor, heated to 500° F. |
|---|--|--|--|--|--|
| In each drop 2 or 3 small black vibrios, moving to and fro. | Abundance of life.   | Abundance of life.   | Much less life than in the two fluids previously examined.   | In each drop 2 or 3 small black vibrios, moving to and fro.  | In each drop 2 or 3 small black vibrios, moving to and fro.  |

These results clearly show that, at the temperatures of 100°, 212°, and 300° F., life and its germs had not been destroyed, whilst at 400° F. they had; for the results of the examination were in this case exactly identical with those of the albumen solution itself; and the life found was doubtless introduced in the preparation of the solution, and was not due to any life having remained in the fluids that had been heated.

Although perfectly aware of the interesting researches of Professor Melsen, proving that the most intense cold does not destroy the active power of vaccine lymph, still I thought it desirable to ascertain the effect of a temperature of 15° F. on well-developed germ-life, similar to that which had been subjected to the action of heat.

Some putrid-meat liquor, therefore, containing a large quantity of microzyma and vibrios, was subjected for twenty hours to the influence of a temperature ranging between the freezing-point of water and 17° below that point, when the ice was melted and the liquor examined. The animalcules retained their vitality, but appeared very languid, and their power of locomotion was greatly decreased.

Two hours after melting the ice the liquor was again examined, when the animalcules appeared to be as energetic as before.

The Society adjourned over Ascension Day, to Thursday, May 25th.